Salivary Peptide P-C Potentiates Insulin Release and Inhibits Glucagon Release from Isolated Perfused Pancreas of the Diabetic GK Rat

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ABSTRACT—We investigated the effects of the salivary peptide P-C (P-C), a saliva-derived peptide, on glucose (8.3 mM)- and arginine (10 mM)-induced insulin release and arginine (10 mM)-induced glucagon release using the perfused pancreas of spontaneously diabetic GK rats. Both its potentiating effect on insulin release and its inhibitory effect on glucagon release were concentration-dependent in diabetic GK rats. The ratio of insulin release obtained with P-C (194 nM) to that without P-C in GK rats was the same as ratio in normal Wistar rats. The ratio (0.40) of glucagon release obtained with P-C (194 nM) to that without P-C was smaller in diabetic GK rats than that (0.75) in normal Wistar rats. These results indicate that P-C inhibits arginine-induced glucagon release in diabetic GK rat pancreas more effectively than in normal Wistar rat pancreas.

Keywords: Salivary peptide P-C, Insulin release, Inhibition of glucagon release, Pancreas (perfused), Diabetic GK rat

Salivary peptide P-C (P-C) isolated from human saliva is a proline-rich polypeptide comprising 44 amino acid residues (1, 2). From immunohistochemical studies, P-C-like immunoreactivity is found in human pancreatic B cells (3), being localized in the insulin secretory granules of these cells (4). A decrease in positive immunofluorescence due to P-C-like immunoreactivity has been reported in the pancreas of diabetic (non-insulin-dependent diabetes mellitus, NIDDM) patients (5, 6). Therefore, a decrease in the level of P-C may accompany the occurrence of diabetes.

In a previous study, we reported that P-C has an anti-hyperglycemic effect on alloxan-induced diabetic mice (7) and that P-C remarkably potentiates glucose (8.3 and 16.7 mM)-induced insulin release and inhibits arginine (10 mM)-induced glucagon release in a concentration-dependent manner in the isolated perfused pancreas of normal Wistar rats (8).

The aim of the present study was to determine whether P-C modulates insulin and glucagon release using the isolated perfused pancreas of GK rats, a spontaneous NIDDM (Type II) model not exhibiting obesity (9–11).

MATERIALS AND METHODS

Male GK (Goto-Kakizaki) rats (18- to 20-weeks-old; body weight, 348–393 g; blood glucose levels, 208–260 mg/dl) were used as the diabetic rats, and they were compared with age-matched normal male Wistar rats (body weight, 474–512 g; blood glucose levels, 92–122 mg/dl), which served as the controls. GK rats (SPF) were donated by Dr. Kenichi Suzuki (Faculty of Medicine, Tohoku University, Sendai) and inbred in our laboratory. Normal Wistar rats were purchased from Sankyo Labo Service (Tokyo). Perfusion of the isolated pancreas was carried out as described previously (8). The perfusate was a basal medium of Krebs-Ringer bicarbonate buffer solution (pH 7.4) containing 0.5% bovine serum albumin (Fraction V; Sigma, St. Louis, MO, USA), 2% dextran (T-70; Pharmacia, Uppsala, Sweden) and 2.8 mM D-glucose (12) saturated with a gas mixture of 95% O₂ and 5% CO₂. The test substances were dissolved in the basal medium at room temperature. The test solutions were introduced into the pancreatic preparation at a constant gas pressure and warmed to 37°C immediately before reaching the pancreas. The flow rate was kept at 1.0 ml/min. Every 1 min, 1.0 ml of perfusate was collected from a portal vein catheter. The pancreas was initially perfused with the

basal medium for 30 min and then perfused with the test solution. Salivary peptide P-C that had the following sequence (H-GRPQGGPPQGGHQQGPPPPPPGKPPQGGP³⁰PPQGGRPQGGPPQGGSPQ-OH) was chemically synthesized by Toray Research Institute (Kamakura). Arginine HCl (L-isomer; Wako, Osaka) was used for secretagogue.

The concentrations of insulin and glucagon in the perfusate collected from a portal vein catheter were determined by radioimmunoassay, as reported previously (13). The results were statistically analyzed with Student's unpaired *t*-test.

RESULTS

Potentiating effect of salivary peptide P-C on glucose- and arginine-induced insulin release in diabetic GK rats

The effect of P-C on the glucose (8.3 mM)- and arginine (10 mM)-induced increase of immunoreactive insulin (IRI) level was investigated in the isolated perfused pancreas of spontaneously diabetic GK rats. The submaximal concentrations were used to avoid the ceiling effect. The concentrations of secretagogues needed to release insulin in the perfused rat pancreas were 8.3 mM of glucose and 10 mM of arginine. Potentiation of glucose-induced IRI level with P-C was concentration-dependent at 97–388 nM of P-C, for repeated 10-min exposures (Fig. 1). The increase in IRI level induced by P-C was not due to the prim-

Fig. 1. Potentiating effect of salivary peptide P-C (P-C, 97–388 nM) on 8.3 mM glucose-induced immunoreactive insulin (IRI) release from perfused pancreas of diabetic GK rat. The columns show the period (10 min) of stimulation by 8.3 mM glucose with P-C (shaded columns) or without P-C (open columns). Closed circles show the IRI level every 1 min. Dotted lines show the end of stimulation and washing with basal medium. Note that P-C potentiated 8.3 mM glucose-induced insulin release in a concentration-dependent manner.

Table 1. Potentiating effect of salivary peptide P-C on 8.3 mM glucose-induced IRI release from the perfused pancreas of diabetic GK rat

Glucose (mM)	P-C (nM)	IRI release (ng/10 min)	Release ratio for glucose
8.3	0	6.85 ± 0.70	1.00
8.3	97	10.42 ± 0.78*	1.52
8.3	194	14.80 ± 1.15**	2.16
8.3	388	16.74 ± 1.01**	2.44

Values are shown as means ± S.E.M. of 4 observations. **P* < 0.05 and ***P* < 0.01, significantly different from the effect of glucose alone by Student's *t*-test.

ing effect by glucose stimulation because repeated stimulation with glucose (16.7 mM) for 10 min released a constant amount of IRI, and the same concentrations of P-C used alone had no effect on the IRI level (8). At 194 nM, P-C had the same potentiating effect on the glucose (8.3 mM)-induced IRI level as the repeated 10-min exposures to glucose. The measured values in Table 1 are presented as the IRI release in response to the secretagogue (glucose alone or glucose with P-C) subtracted by the basal release. The insulin-releasing effect is represented as the ratio of the IRI level with P-C and glucose (8.3 mM) to that without P-C (8.3 mM glucose alone).

Fig. 2. Typical data for the effect of 194 nM P-C on 8.3 mM glucose-induced IRI release from perfused pancreas of diabetic GK rat (solid lines) and normal Wistar rat (broken line). The glucose stimulation was continuous from 0 to 30 min after simultaneous administration with P-C (closed circles) or without P-C (open circles). The shaded area represents the difference between the glucose-induced IRI release by P-C and the control value without P-C. Note that the impaired insulin response to glucose (8.3 mM) stimulation in diabetic GK rats is significantly improved by P-C, especially in the first phase.

Basal IRI release in the basal perfusion medium (2.8 mM glucose) was significantly ($P < 0.01$) higher in GK rats (2.35 ± 0.26 ng/ml, $n = 26$) than in normal Wistar rats (0.52 ± 0.14 ng/ml, $n = 27$). After preperfusion for 30 min with the basal medium, the continuous 30-min infusion of 8.3 mM glucose provoked a biphasic pattern of IRI release that consisted of a rapid first peak and a second phase of gradual increase in the normal pancreas. In the pancreas of diabetic GK rats, the immediate first phase of release was obviously lowered, and the second phase was generated very slowly throughout the 30-min stimulation. The total amount of IRI release in response to glucose in GK rats (27.8 ± 2.9 ng/30 min, $n = 8$) was markedly lower compared to that in normal Wistar rats (85.3 ± 6.4 ng/30 min, $n = 8$). As the submaximal response of insulin release was obtained at 194 nM of P-C (Table 1), the insulin-releasing effects were observed continuously from 0 to 30 min after the administration of 194 nM P-C with glucose (Fig. 2) and arginine (Fig. 3), respectively. The suppression of glucose-induced insulin release in the diabetic state was significantly reversed by 194 nM P-C. The early peak of release became clearly detectable, although the second phase was still slowly increased. The total amount of IRI release with P-C was increased 1.73-fold in the GK rat pancreas and by 1.71-fold in the normal Wistar rat pancreas, as compared with that without P-C (glucose alone).

The biphasic response of the arginine (10 mM)-induced

Fig. 3. Typical data for the effect of 194 nM P-C on 10 mM arginine-induced IRI release from perfused pancreas of GK rats (solid lines) and normal Wistar rats (broken line). The arginine stimulation was continuous from 0 to 30 min after simultaneous administration with P-C (closed circles) or without P-C (open circles). The shaded area represents the difference between the arginine-induced IRI release by P-C and the control value without P-C. Note that P-C potentiates the arginine (10 mM)-stimulated increase in both phases of insulin release in diabetic GK rats.

insulin release in diabetic GK rats was enhanced in appearance (Fig. 3). However, when the high basal IRI level of the diabetic state was subtracted from the arginine-induced release, the net amount of arginine-induced IRI release in GK rats (57.5 ± 5.5 ng/30 min, $n = 7$) was not significantly different from that in normal Wistar rats (63.5 ± 3.1 ng/30 min, $n = 8$). P-C potentiated both the first and second phases of the arginine-induced increase in IRI level in GK rats. The total amount of IRI release with P-C was increased by 1.53-fold in GK rat pancreas and by 1.62-fold in normal Wistar rats, as compared with that without P-C (arginine alone).

Inhibitory effect of salivary peptide P-C on arginine-induced glucagon release in diabetic GK rat

We investigated the effect of P-C on the arginine (10 mM)-induced decrease in immunoreactive glucagon (IRG) level from the isolated perfused pancreas of spontaneously diabetic GK rats. P-C at 194–388 nM significantly inhibited the amount of IRG released by 10 mM arginine. Inhibition of arginine-induced IRG level by P-C was concentration-dependent for repeated 10-min exposures to P-C (Fig. 4). The decrease in IRG level induced by P-C was not due to the priming effect by arginine stimulation because repeated arginine (10 mM) stimulation for 10 min releases a constant amount of IRG, and the same concentrations of P-C used alone have no effect on the IRG level (data not shown). The measured values in Table 2 are presented as the IRG release in response

Fig. 4. Inhibitory effect of P-C (97–388 nM) on 10 mM arginine-induced immunoreactive glucagon (IRG) release. The columns show the period (10 min) of stimulation by 10 mM arginine with P-C (shaded columns) or without P-C (open columns). Closed circles show the IRG level every 1 min. Dotted lines show the end of stimulation and washing with basal medium. Note that P-C inhibited 10 mM arginine-induced glucagon release in a concentration-dependent manner.

Table 2. Inhibitory effect of salivary peptide P-C on 10 mM arginine-induced IRI release from the perfused pancreas of diabetic GK rat

Arginine (mM)	P-C (nM)	IRG release (ng/10 min)	Release ratio for arginine
10	0	6.42 ± 0.93	1.00
10	97	5.34 ± 0.74	0.83
10	194	3.65 ± 0.68*	0.57
10	388	3.27 ± 0.69*	0.51

Values are shown as means ± S.E.M. of 3 observations. * $P < 0.05$, significantly different from the effect of arginine alone by Student's *t*-test.

to the secretagogue (arginine alone or arginine plus P-C) subtracted by the basal release. The glucagon-decreasing effect is represented as the ratio of the IRI level with P-C to that without P-C (10 mM arginine alone).

After preperfusion for 30 min with the basal medium, the continuous 30-min infusion of 10 mM arginine provoked an immediate first peak of IRI release in diabetic GK rats, and the total amount of IRI release (22.3 ± 3.2 ng/30 min, $n = 7$) was very low compared with that in normal Wistar rats (137.1 ± 5.7 ng/30 min, $n = 8$). As the glucagon-decreasing response was submaximal at 194 nM

Fig. 5. Typical data for the effect of 194 nM P-C on 10 mM arginine-induced IRI release from the perfused pancreas of GK rats (solid lines) and normal Wistar rats (broken line). Arginine stimulation was continuous from 0 to 30 min after simultaneous administration with P-C (closed circles) or without P-C (open circles). The shaded area represents the difference between the arginine-induced IRI level by P-C and the control value without P-C. Note that the impaired glucagon response to arginine (10 mM) stimulation in diabetic GK rats is further inhibited by P-C.**Table 3.** Effects of salivary peptide P-C (194 nM) on glucose- and arginine-induced IRI release and arginine-induced IRI release during 30 min from the isolated perfused pancreas of normal Wistar and diabetic GK rats

		Normal Wistar rat		GK rat	
IRI release (ng/30 min)	Glucose (8.3 mM)	85.3 ± 6.4 ^{a)}		27.8 ± 2.9	
	Glucose + P-C	145.8 ± 12.0		48.2 ± 9.3	
	Release ratio	1.71 ^{b)}		1.73	
	Arginine (10 mM)	63.5 ± 3.1		57.5 ± 5.5	
	Arginine + P-C	103.1 ± 5.9		88.2 ± 4.9	
IRG release (ng/30 min)	Release ratio	1.62 ^{b)}		1.53	
	Arginine (10 mM)	137.1 ± 5.7		22.3 ± 3.2	
	Arginine + P-C	102.3 ± 8.4		8.9 ± 1.3	
		0.75 ^{c)}		0.40	

^{a)}Values are shown as means ± S.E.M. of 4–8 observations. Values for the normal Wistar rats are the means ± S.E.M. of both age-matched (18–20 weeks) and non age-matched (6–7 weeks) controls, because each value of both controls was the same. All values were obtained by subtracting the basal values (2.8 mM glucose) without glucose (8.3 mM), arginine and P-C. ^{b, c)}Values indicate ratio changes for glucose- and arginine-induced IRI release and arginine-induced IRI release without P-C. Data are partly from ref. 8. ** $P < 0.01$, significantly different by Student's *t*-test.

P-C (Table 2), the time course of IRG release was measured continuously for 30 min after the administration of 194 nM P-C with arginine (Fig. 5). The arginine-induced IRG release was reduced by P-C. The total amount of IRG release in the presence of P-C was 0.4 times lower in GK rat pancreas and 0.75 times lower in normal Wistar rats as compared with the respective levels without P-C (arginine alone, Table 3). The inhibitory effect by P-C on arginine-induced glucagon release was concentration-dependent in GK rats. P-C is more effective in inhibiting arginine-induced glucagon release in the pancreas of diabetic GK rats than in that of normal Wistar rats (Table 3).

DISCUSSION

The growth and function of the parotid gland are mainly regulated by insulin *in vivo*, and a significant reduction of parotid gland DNA, RNA and total protein content is produced by diabetes mellitus (14). The parotid gland may be the source of an insulinotropic principle that maintains the sensitivity of pancreatic B cells to circulating glucose (15). Therefore, the findings in this study suggest a close endocrine relationship between the salivary glands and the pancreas in the diabetic state. P-C derived from human saliva is localized in the insulin-secretory granules of pancreatic B cells (4). However, it has not been determined whether there is a direct link between saliva and pancreatic B cells. A decrease in P-C-like immunoreactivity is frequently observed in the pancreas of patients with NIDDM (5, 6). The above findings suggest that the decrease in the level of P-C may be a significant preliminary signal for the occurrence of diabetes. Furthermore, we have reported that P-C has an anti-hyperglycemic effect on alloxan-induced diabetic mice (7) and that only at high concentrations of glucose (8.3 mM and above) does P-C potentiate insulin release in the perfused pancreas of normal Wistar rats (8). We speculate that hyperglycemia may be caused partly by a deficiency of P-C.

This paper clarified that P-C simultaneously regulates both insulin and glucagon release using the pancreas of spontaneously diabetic GK rats. The GK rat is a useful model for studying the etiology of B cell desensitization to glucose (16). The GK rat, established from the Wistar rat, is a non-obese model of spontaneous NIDDM (9–11). Resting plasma insulin levels in GK rats are somewhat higher than those in normal Wistar rats (16). Low (5.6 mM) and high (20 mM) glucose-stimulated insulin release from the isolated pancreas is higher and lower, respectively, in GK rats than in normal Wistar rats (17). Arginine (10 mM) immediately provokes the first phase of glucagon release in GK rats (18). Deficient levels of P-C have not been measured in GK rats, but are found in patients with human NIDDM (5, 6). We summarize our

present results as well as previous (8) data in Table 3. Glucose-induced insulin release and arginine-induced glucagon release were remarkably suppressed in the diabetic state, although the arginine-induced insulin release was not altered by the diabetic state. In diabetic GK rats, P-C induced a concentration-dependent recovery in the first phase of glucose-induced insulin release and potentiated it for the arginine-induced release. P-C also potentiated both the second phases of insulin release induced by glucose and arginine. Therefore, P-C selectively stimulated B cells and improved the impaired insulin response to glucose in the spontaneously diabetic rat. Furthermore, under the diabetic state, P-C inhibited glucagon release in a concentration-dependent manner, in the same concentration range as that used to improve insulin release. The improvement in insulin release induced by P-C was the same in the normal and diabetic state. However, the inhibition of glucagon release by P-C was more remarkable in the diabetic state than in the normal state (Table 3).

As an intracellular second messenger, Ca^{2+} may act as an initiator of glucagon release, whereas cyclic AMP and the activation of protein kinase C may modulate release, and ATP is required for glucagon release (19). Diacylglycerol and Ca^{2+} may be involved in mediating the effect of arginine on glucagon release in A cells, although such an involvement is not apparent in arginine-stimulated B cells (20). Furthermore, islet hormone release is regulated by intra-islet intercellular interactions via capillary vessels and/or paracrine routes. The intra-islet interactions depend on the direction of flow in islet vascular perfusion from B cells to A cells by anterograde and retrograde infusion of islet hormone antibody (21). P-C selectively stimulates B cells to potentiate insulin release, and then may inhibit glucagon release via the vascular route in diabetic pancreas. Insulin inhibits the release of glucagon (22), whereas glucagon directly stimulates insulin release (23). The mechanisms by which P-C induces insulin release and inhibits glucagon release seem to be independent of each other. This may be supported by the fact that the ratio of glucagon release was inhibited more markedly in the diabetic state than in the normal state. As a result of regulation in the endocrine pancreas, several peptides such as P-C or neurotransmitter may contribute to the secretory process.

In conclusion, P-C may regulate both glucose- and arginine-induced insulin release and arginine-induced glucagon release from the perfused pancreas. P-C inhibits the arginine-induced glucagon release in the pancreas of diabetic GK rats more effectively than in that of normal Wistar rats. Thus P-C is involved in the improvement of hyperglycemia in spontaneously diabetic GK rats.

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